A DYNAMICAL THEORY OF ANTAGONISM.

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(Read April 14, 1916.)

When toxic substances act as antidotes to each other we call this action antagonism. The writer has found that an accurate measure of antagonism is furnished by the electrical resistance of living tissues. A toxic substance causes a fall of resistance: but if another toxic substance be added and the fall of resistance be thereby inhibited it is evident that this result is due to antagonism. The amount of antagonism may therefore be measured by the fall of resistance.

A series of such measurements is reported in the present paper.[1] The method of making these measurements has been previously described.[2]

The experiments consisted in determining the electrical resistance of *Laminaria Agardhii*[3] in NaCl .52 M, in $CaCl_2$ .278 M and in various mixtures of these.

In order that each solution might contain as nearly as possible the same kind of material the following method was employed. Seven disks were cut (by means of a large cork borer) from the same part of a frond: each disk was placed in a separate tumbler of sea water. A second lot of seven disks were cut, as close to each other as possible, and placed in the tumblers, so that each tumbler contained two disks. This was continued until each tumbler con-

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[1] In an earlier series (Pringsheim's *Jahrb. für wiss. Bot.,* 54: 645, 1914) the maximum antagonism was found in mixtures containing less NaCl than is here reported. The present series comprises six sets of experiments (in the earlier there were only three), was made at more nearly constant temperature and with an improved technique. The later results may therefore be regarded as more reliable.

[2] *Science,* N. S., 35: 112, 1912.

[3] This is the most common species at Wood's Hole, Mass.; it was formerly identified as *L. saccharina* and is so referred to in earlier papers by the writer.

tained one hundred disks. By this means the material in the different tumblers was made as similar as possible. The disks in each tumbler were then packed together (like a roll of coins) to form a cylinder whose resistance was measured. Throughout the experiments the different lots were kept side by side and treated as nearly alike as possible, except that they were placed in different solutions.

Great care was taken that all the solutions (both pure and mixed) should have the same conductivity as sea water.

The distilled water used in making the solutions was prepared with especial care, the first and last part of the distillate being discarded. It was distilled from a glass still (which had been used for some months) using plugs of cotton in place of cork or rubber stoppers.

The salts used were the purest obtainable, being for the most part Kahlbaum's (in some cases Merck's blue label reagents were employed).

The mixtures were made in the following proportions:

NaCl .52 M, c.c.	CaCl$_2$ .278 M, c.c.	Molecular Proportions.	
		NaCl.	CaCl$_2$.
963	37	98	2
914	86	95.24	4.76
751	249	85	15
496	504	65	35
247	753	38	62

The results[4] are shown graphically in Fig. 1 (cf. Table III.). As will be seen on inspection of the figure, the resistance rises at first (except in pure NaCl) and subsequently falls.

Evidently two processes are involved, one of which produces a rise, the other a fall in resistance. While these processes might be looked upon as independent, everything points to the fact that they are casually connected, and it seems natural to assume that they represent two chemical reactions, one of which is dependent on the other.

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[4] The results are expressed as per cent. of the net resistance in sea water at the start of the experiment. They might also be expressed as per cent. of the control but this has no especial advantage for the present investigation.

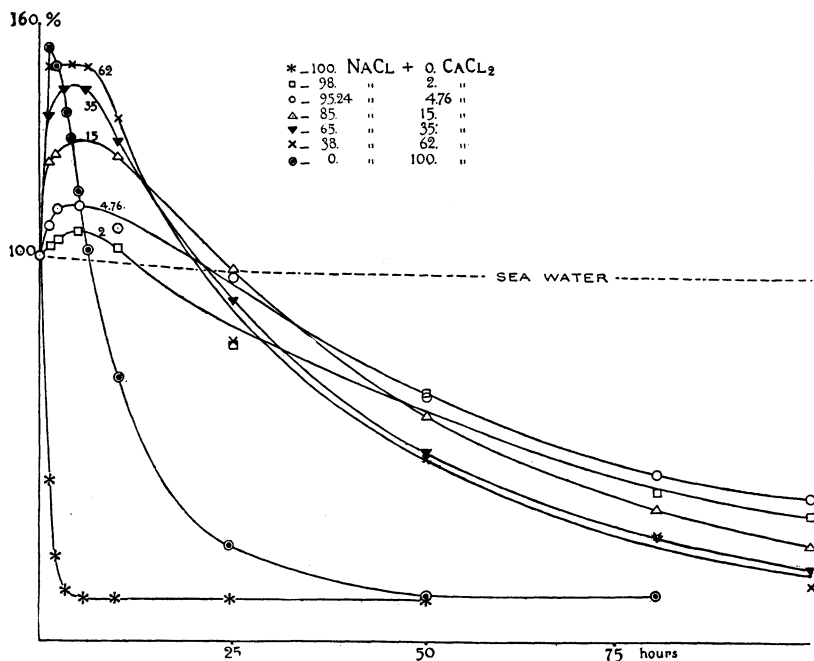We may suppose for the sake of simplicity that they represent two monomolecular reactions[5] of the type

$$A \to M \to B,$$

FIG. 1. Curves of electrical resistance of *Laminaria* in NaCl .52 M, in CaCl$_2$ .278 M, and in mixtures of these (the figures show the molecular per cent. of CaCl$_2$ in the mixture). Some points are omitted in order to avoid undue crowding (these may be obtained from Table III).

in which a substance A breaks down into an intermediate substance M which in turn breaks down to form B. In such a reaction the amount of M at first increases, reaches a maximum and then decreases.

The nature of this process is evident from a consideration of Fig. 2. If the reservoir A be filled with water while M and B are empty, and if water be allowed to flow from A into M, the amount of water in M (for convenience this amount is called y) will first

[5] Or other reactions of the first order as for example when two substances react but one is present in great excess.

increase and then decrease. The rate of increase and decrease and the maximum attained will depend on the relation between the two outlets K_1 and K_2. We may suppose that if K_1 is equal to K_2 we get the upper curve shown in the figure, while if K_1 is less than K_2 we get the lower curve (in the latter case both constants are supposed to be smaller than in the former). This is analogous to what occurs in the reaction $A \rightarrow M \rightarrow B$ if K_1 is the velocity constant of $A \rightarrow M$ and K_2 is the velocity constant of $M \rightarrow B$.
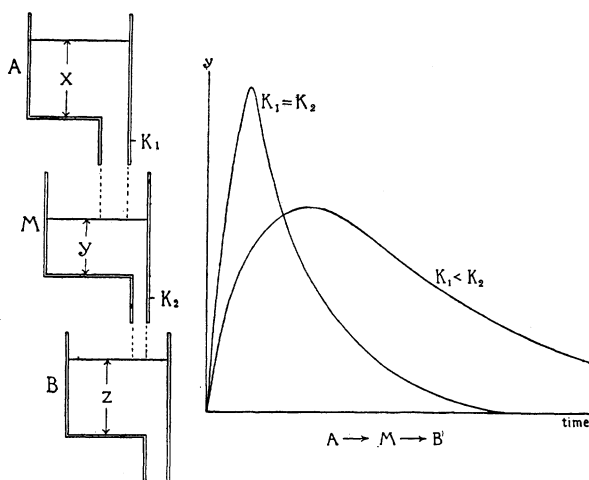

Fig. 2. Diagram illustrating consecutive reactions in which a substance M is formed by the reaction $A \rightarrow M$ and decomposed by the reaction $M \rightarrow B$. Explanation in text.

We find that the resistance of the tissue likewise increases to a maximum and then decreases. If we assume that the resistance of the protoplasm is due to a substance[6] M we can calculate the rate at which the resistance will increase and decrease with any given values of K_1 and K_2.

If we suppose that in sea water the substance M results from the decomposition of A, which is in turn formed at a constant rate, the amount of M will be constant (after equilibrium has once been

[6] Instead of a substance we might assume that M is a physical state or a mixture provided it fulfilled the necessary conditions of formation and disappearance.

attained). For convenience we assume that at equilibrium the concentration of M (which we call y) is equal to 0.2951 and that the net resistance (expressed as per cent. of the net resistance in sea water) is equal to $305y + 10$, the 10 being added because at death the resistance drops to 10 per cent. (*i. e.*, the resistance of the dead tissue is 10 per cent. of that of the living). The resistance in sea water will therefore be $(.2951 \times 305) + 10 = 100$ per cent.

Let us assume that the velocity constant of the decomposition of M in sea water is .540. If the velocity constant of the reaction $A \rightarrow M$ is one thirtieth as great $(.540 \div 30 = .018)$ the concentration of A must be 30 times as great as that of M in order to keep M constant. Hence the concentration of A (which will be called x) must be $.2951 \times 30 = 8.853$.

On transferring from sea water to such a mixture as 65 NaCl + 35 $CaCl_2$ we assume that the production of A ceases while the decomposition of A and M go on at an altered rate, the velocity constant for $A \rightarrow M$ being changed to .000481 and that of $M \rightarrow B$ to .00859. We can now calculate the value of y at any subsequent time, T.

The value of y at the start is .2951 and this will decrease (by the ordinary monomolecular formula) in the time T to

$$y = .2951(e^{-K_2 T}).$$

In this formula e is the basis of natural logarithms and K_2 is the velocity constant of the reaction $M \rightarrow B$, which results in the decomposition of y.

The value of x at the start is 8.853: this will produce during the time T a certain amount of y part of which will be decomposed. The amount remaining at the end of the time T is given by the formula[7]

$$y = .8.853 \left(\frac{K_1}{K_2 - K_1} \right) (e^{-K_1 T} - e^{-K_2 T}),$$

[7] Rutherford, E., "Radioactive Substances and their Radiations," 1913, p. 421. The values $e^{-K_1 T}$ and $e^{-K_2 T}$ may be obtained from Table IV. in the Smithsonian Mathematical Tables, Hyperbolic Functions, by G. F. Becker and C. E. Van Orstrand, 1909. See also Mellor, J. W., "Chemical Statics and Dynamics, 1909, pp. 16, 98, 118.

in which K_1 is the velocity constant of the reaction $A \rightarrow M$. The total value of y at the time T will be

$$y = .2951(e^{-K_2 T}) + 8.853 \left(\frac{K_1}{K_2 - K_1} \right) (e^{-K_1 T} - e^{-K_2 T}).$$

If we put $K_1 = .000481$ and $K_2 = .00859$ we obtain as the amount of resistance the values given in Table III. and plotted as a broken line in Fig. 3 (the values obtained experimentally are plotted as a continuous line).
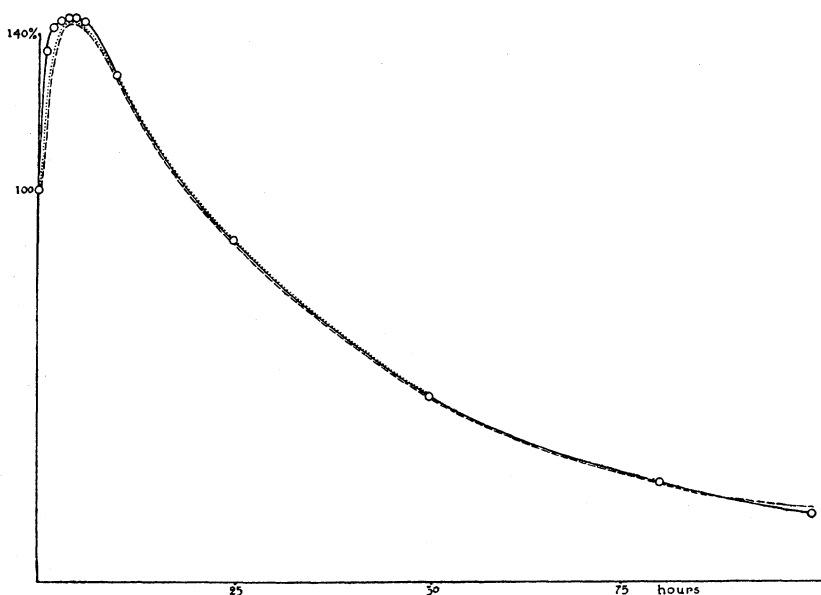

FIG. 3. Curve of electrical resistance of *Laminaria* in 65 NaCl + 35 CaCl₂ (————), the trial curve (— — — — —) calculated from the velocity constants $K_1 = .000481$ and $K_2 = .00859$, and the theoretical curve (..........) calculated from the velocity constants $K_1 = .000482$ and $K_2 = .00859$.

Evidently the curve of resistance,[8] as calculated, agrees[9] fairly

[8] The agreement in other mixtures may be seen in Table III. and Figs. 6-9.

[9] The fact that the theoretical curves can not be made to assume any desired form but can only be varied within certain limits shows that if they fit the experimental curves they express processes similar to those which take place in the tissue.

well with the curve of resistance in 65 NaCl + 35 $CaCl_2$ obtained experimentally.

We see that in 65 NaCl + 35 $CaCl_2$ the resistance of the tissue behaves as if it were directly proportional to y (the amount of M) when the velocity constant of $A \rightarrow M$ is .000481 and the velocity constant of $M \rightarrow B$ is .00859. We may interpret this to mean that some substance, A, in the protoplasm breaks down to form M, the substance to which the resistance of the protoplasm is due, and this is in turn decomposed, the two reactions having the velocities indicated.

By making this very reasonable assumption we can account for all the curves of resistance obtained experimentally.

In order to see how this may be accomplished we may calculate the constants of all the curves, proceeding in the same manner as in calculating the constants of the curve of 65 NaCl + 35 $CaCl_2$. For this purpose we assume various values until the correct ones are discovered. The values obtained by this method are given in Table I.

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TABLE I.

RELATION OF K_2 TO Na_2XCaCl_4.

Constants obtained by trial.

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Molecular Proportions.				Amount of Na_2-$XCaCl_4$.	Increase of Na_2-$XCaCl_4$.	K_1.	K_2.	Decrease of K_2.	(Decrease of K_2) ÷ (Increase of Na_2XCaCl_4).
In Body of Solution.		In Surface.							
NaCl, %.	$CaCl_2$, %.	NaCl, %.	$CaCl_2$, %.						
98	2	83.05	16.95	.0001169	.0001137	.000253	.00672	.00228	19.35
95.24	4.76	66.67	33.33	.0001468	.0001436	.000245	.00590	.00310	21.59
85	15	36.27	63.73	.0000839	.0000807	.000364	.00730	.00170	21.07
65	35	15.66	84.34	.0000207	.0000175	.000481	.00859	.00041	23.43
38	62		5.78	94.22	.0000032		.000530	.00900	
100	0					.018	.540		
0	100					.0018	.0295		

An inspection of Table I. shows that as the amount of $CaCl_2$ increases, the value of K_2 first falls and then rises, the minimum value occurring at 95.24 NaCl + 4.76 $CaCl_2$ (which is the mixture in which the tissue lives longest). It is evident that in each mixture of NaCl + $CaCl_2$ a substance is formed which in some way reduces

the value of K_2. We may assume that the amount of this substance increases as $CaCl_2$ increases and that its maximum effect on K_2 is produced in 95.24 NaCl + 4.76 $CaCl_2$, after which a further increase of this substance produces less and less effect on K_2. Or we may assume that the decrease of K_2 is directly proportional to the amount of this substance but that the maximum amount of this substance is produced in 95.24 NaCl + 4.76 $CaCl_2$, while in other mixtures it is produced in lesser amounts. Let us consider more fully the latter alternative.

The simplest assumption which we can make is that both NaCl and $CaCl_2$ combine with some substance, X, in the protoplasm so as to form a compound. If we suppose that this compound[10] is $Na_{20}XCaCl_{22}$ formed by the reversible reaction

$$20NaCl + X + CaCl_2 \rightleftharpoons Na_{20}XCaCl_{22}$$

we can calculate the amount of $Na_{20}XCaCl_{22}$ which will be formed in each mixture of NaCl + $CaCl_2$.

The molecular concentration of X can hardly be more than a small fraction of that of NaCl and $CaCl_2$. Hence as NaCl and $CaCl_2$ are present in great excess they may be regarded as constant in concentration and we need only consider the changes in X and $Na_{20}XCaCl_{22}$.

We may calculate the amounts of $Na_{20}XCaCl_{22}$ formed at equilibrium in any mixture by means of the formula

$$K = \frac{Conc_{Na_{20}XCaCl_{22}}}{(Conc_{NaCl})^{20}(Conc_{CaCl_2})(Conc_X)},$$

in which K is a constant and the molecular concentrations of $Na_{20}XCaCl_{22}$, NaCl, $CaCl_2$, and X are denoted by the abbreviation Conc. We find that the amount of $Na_{20}XCaCl_{22}$ begins with o in pure NaCl, increases with the increase of $CaCl_2$ until it reaches a maximum at NaCl 95.24 + $CaCl_2$ 4.76 and then decreases (as $CaCl_2$ continues to increase) until it falls to o in pure $CaCl_2$. As this is exactly what K_2 does, we might assume that $Na_{20}XCaCl_{22}$ acts as a negative catalyzer, causing a decrease in K_2 which is directly proportional to the amount of $Na_{20}XCaCl_{22}$.

[10] Such compounds are frequently formed between salts and proteins as well as with many other amphoteric electrolytes.

To this however there is the objection that the maximum amount of $Na_{20}XCaCl_{22}$ which is produced in NaCl $95.24 + CaCl_2$ 4.76 could also be obtained in other mixtures, *e. g.,* in NaCl 50 + $CaCl_2$ 50 if the total concentration of salts in the latter mixture were increased to the requisite amount. This is contrary to what we find in antagonism experiments. If antagonism really depends on the production of a salt compound like $Na_{20}XCaCl_{22}$ it is evident that some mechanism must exist which insures that an increase in the total concentration of salts can have but little effect as compared with that produced by a change in their relative proportions.

It is easy to see how such a mechanism must exist if the formation of $Na_{20}XCaCl_{22}$ takes place at a surface (at the external surface of the cell or at internal surfaces). In a surface substances usually exist in a different concentration from that which they have elsewhere in the solution. If NaCl and $CaCl_2$ migrate into the surface. so as to become more concentrated there than in the rest of the solution, their concentration in the surface must increase as their concentration in the solution increases until a certain point (called the saturation point) is reached. Beyond this point an increase in their concentration in the solution produces no effect on their concentration in the surface.

When this stage has been reached the formation of $Na_{20}XCaCl_{22}$, if it takes place in the surface, will not be affected by an increase in the concentration of the salts in the solution. It will, however, be affected by changes in the relative proportions of the salts. The number of molecules of salt in a unit of surface will remain nearly constant, but if the proportion of NaCl in the solution be increased some of the $CaCl_2$ in the surface will be displaced by NaCl.

Below the saturation point the relative proportions of the salts will be of less importance than their total concentration: this is the case at low concentrations in the region of the so-called "nutritive effects."

It may be added that these considerations apply no matter whether the salts which migrate into the surface come from the outside or from within (or from both directions): also that they apply when the surface is completely permeable to salts (so that we do not assume an impermeable surface in order to account for

the formation of the salt compound which itself accounts for the permeability). They also apply if $Na_{20}XCaCl_{22}$ is formed elsewhere than at the surface and subsequently migrates into the surface for, as the reaction is reversible, the equilibrium in the surface must be determined by the amounts of X, NaCl, and $CaCl_2$, regardless of whence they are derived.

Let us consider what will happen if NaCl and $CaCl_2$ do not migrate equally into the surface. If for example $CaCl_2$ accumulates in the surface ten times as much[11] as NaCl we shall have in the surface NaCl $2 + CaCl_2$ 1 when the body of the solution contains NaCl $20 + CaCl_2$ 1 (or NaCl $95.24 + CaCl_2$ 4.76).
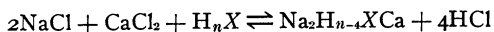
Let us assume that this is the case and that the salt compound formed is not $Na_{20}XCaCl_{22}$ but Na_2XCaCl_4. Its maximum amount will be produced when the solution contains NaCl $20 + CaCl_2$ 1 or NaCl $95.24 + CaCl_2$ 4.76 and will be independent of the total concentration of salts so long as the surface is saturated. We may calculate the amounts present in the surface according to the formula.[12]

$$K = \frac{Conc_{Na_2XCaCl_4}}{(Conc_{NaCl})^2(Conc_{CaCl_2})(Conc_X)}.$$

When we have NaCl $95.24 + CaCl_2$ 4.76 in the solution we have in the surface NaCl $9.524 + CaCl_2$ 4.76 or NaCl 66.67 per cent. $+ CaCl_2$ 33.33 per cent.; and since they are present in excess this will

[11] There is experimental evidence in favor of the view that $CaCl_2$ accumulates in the surface more than NaCl.

[12] If we write

$$2NaCl + CaCl_2 + H_nX \rightleftharpoons Na_2H_{n-4}XCa + 4HCl$$

we have at equilibrium

$$K = \frac{(Conc_{Na_2H_{n-4}XCa})(Conc_{HCl})^4}{(Conc_{NaCl})^2(Conc_{CaCl_2})(Conc_{H_nX})}$$

but

$$Conc_{HCl} = 4(Conc_{Na_2H_{n-4}XCa})$$

so that we may write

$$K = \frac{(Conc_{Na_2H_{n-4}XCa})^5}{(Conc_{NaCl})^2(Conc_{CaCl_2})(Conc_{H_nX})}$$

In the present case this assumption does not fit the facts as well as the one already made.

be their concentration at equilibrium (as well as during the progress of the reaction). Putting $K = .000001$ and $\text{Conc}_X = .001$ we get

$$.000001 = \frac{\text{Conc}_{\text{Na}_2X\text{CaCl}_4}}{(66.67)^2(33)(.001)},$$

whence

$$\text{Conc}_{\text{Na}_2X\text{CaCl}_4} = .0001468.$$

Calculating the amounts formed in other mixtures we get the values given in Table I.

FIG. 4. Curve of the increase of a salt compound, $\text{Na}_2X\text{CaCl}_4$ (————), and curve of the decrease of the velocity constant K_2 (— — — —). The figures apply to the ordinates of the former curve. The figures for the ordinates of the latter curve (— — — —) are 21.59 times as great. The abscissæ represent molecular proportions in the solution (not in the surface). The figure shows that the salt compound inhibits the reaction $M \rightarrow B$ (which has the velocity constant K_2).

It is evident from an inspection of the last column of Table I. that the decrease in K_2 is directly proportional to the amount of

Na_2XCaCl_4 (see Fig. 4).[13]   Hence we assume that Na_2XCaCl_4 acts as a negative catalyzer to the reaction $M \to B$.

In some instances the optimum proportion in antagonism experiments turns out to be NaCl 100 + $CaCl_2$ 1.   In this case we should, following the course just outlined, assume that the salt compound is $Na_{10}XCaCl_{12}$.   It may be objected that this calls for a reaction of a high order, the occurrence of which seems not very probable. But it must be remembered that the reaction may take place by a series of steps each of which represents a reaction of the second or third order.   Such cases are well known in inorganic chemistry. In the calculations all these steps may be disregarded since only the final equilibrium need be considered.

An inspection of Table I. shows that the value of K_1 rises and falls with that of K_2 except that as $CaCl_2$ increases the value of K_1 goes up more rapidly than that of K_2.   This is also obvious from Fig. 1, which shows that the greater the per cent. of $CaCl_2$ in the mixture the greater the maximum attained.   As this maximum de-

TABLE II.

RELATION BETWEEN $(K_1 \div K_2)$ AND PER CENT. OF $CaCl_2$ IN SURFACE.
Constants obtained by trial.

| Molecular Proportions. | | | | K_1. | K_2. | $K_1 \div K_2$. | Increase of $K_1 \div K_2$. | (Increase of $K_1 \div K_2$) ÷ (Per Cent. of $CaCl_2$ in Surface). |
| In Solution. | | In Surface. | | | | | | |
NaCl.	$CaCl_2$.	NaCl.	$CaCl_2$.					
100	0	100	0	.018	.540	.03333		
98	2	83.05	16.95	.000253	.00672	.03765	.00432	.000255
95.24	4.76	66.67	33.33	.000245	.00590	.04153	.00820	.000246
85	15	36.27	63.73	.000364	.00730	.04986	.01653	.000259
65	35	15.66	84.34	.000481	.00859	.05600	.02267	.000269
38	62	5.78	94.22	.000530	.00900	.05889	.02556	.000271
0	100	0	100	.0018	.0295	.06102	.02769	.000277

pends on the value of $K_1 \div K_2$ it is obvious that the value of $K_1 \div K_2$ must increase as the per cent. of $CaCl_2$ increases.   We therefore assume that the value of K_1 (like that of K_2) depends on the amount of Na_2XCaCl_4 but that (unlike that of K_2) it is also

[13] The figure makes the curve of Na_2XCaCl_4, bend more abruptly at the apex than is actually the case, but it is so drawn because it is not desirable to use more points in the calculated than in the experimental curve.

dependent on the per cent. of $CaCl_2$ present in the surface. If this assumption is correct the value of $K_1 \div K_2$ will be proportional to the per cent. of $CaCl_2$. This is actually the case, as is evident from Table II. and Fig. 5.
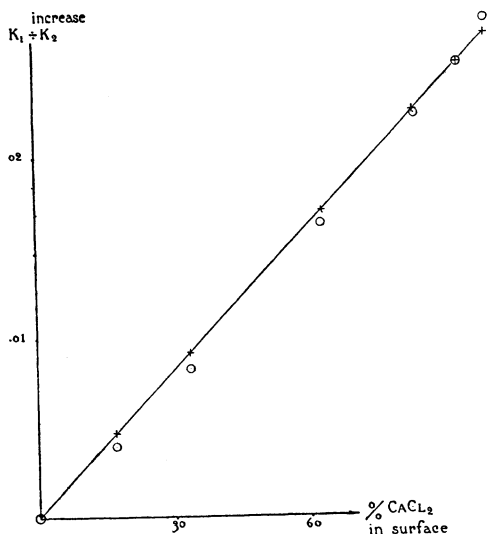

FIG. 5. Curve of the increase of $K_1 \div K_2$ with increase of $CaCl_2$ (molecular per cent. in the surface). The figure shows that $CaCl_2$ acts as a catalyzer of the reaction $A \rightarrow M$ (which has the velocity constant K_1).

We must therefore conclude that the reaction $A \rightarrow M$ is catalyzed by $CaCl_2$, while the salt compound Na_2XCaCl_4 inhibits both $A \rightarrow M$ and $M \rightarrow B$. This assumption enables us to calculate the resistance at any time in any mixture. The calculated results are given in Table III. together with the observed values. The calculations are made on the assumption that the decrease of K_2 is exactly proportional to the increase of Na_2XCaCl_4, that is

$$\frac{\text{decrease of } K_2}{\text{increase of } Na_2XCaCl_4} = 23.29$$

and

$$\frac{\text{increase of } (K_1 \div K_2)}{\text{per cent. } CaCl_2 \text{ in surface}} = .000271.$$

TABLE III.

OBSERVED VALUES COMPARED WITH VALUES CALCULATED ON A THEORETICAL BASIS.

Time in Hours.	Per Cent. of Net Resistance in									
	98 NaCl + 2 CaCl$_2$.		95.24 NaCl + 4.76 CaCl$_2$.		85 NaCl + 15 CaCl$_2$.		65 NaCl + 35 CaCl$_2$.		38 NaCl + 62 CaCl$_2$.	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
1	103.1	103.7	108.2	106.8	124.5	115.6	136.1	123.8	148.1	127.2
2	103.8	105.8	112.1	111.2	126.1	124.8	141.9	136.3	149.0	141.7
3	105.8	106.7	112.1	113.8	128.5	129.8	143.2	142.2	149.0	147.9
4	106.1	106.9	113.9	115.3	130.2	132.1	143.9	144.0	149.0	149.7
5	106.1	106.6	113.1	115.8	130.2	132.7	143.9	143.6	149.0	148.8
6	104.9	106.0	113.1	115.8	130.2	132.3	143.7	141.7	149.0	146.5
10	102.1	101.8	107.9	112.5	126.5	125.1	129.5	129.8	136.1	132.6
25	76.89	84.21	95.21	93.41	96.40	93.80	87.85	87.90	78.21	86.30
50	63.90	61.70	62.50	68.20	58.11	58.83	47.81	47.80	46.34	44.46
80	38.90	43.49	42.52	47.78	33.92	35.54	26.01	25.88	27.11	23.27
100	31.80	35.09	35.83	38.33	24.01	26.58	17.33	18.90	14.42	17.03

The measurements were made at 15° C. or corrected to this figure. Each experimental figure is the average obtained from 6 series of experiments.

The constants obtained by this method of calculation are given in Table IV.

Table III. shows that the agreement between calculated and observed values is remarkably satisfactory.

TABLE IV.

THEORETICAL CONSTANTS.

Molecular Proportions.				K$_1$.	K$_2$.
In Solution.		In Surface.			
NaCl.	CaCl$_2$.	NaCl.	CaCl$_2$.		
100	0	100	0	.018	.540
98	2	83.05	16.95	.000241	.00635
95.24	4.76	66.67	33.33	.000240	.00566
85	15	36.27	63.73	.000360	.00712
65	35	15.66	84.34	.000482	.00859
38	62	5.78	94.22	.000530	.00900
0	100	0	100	.0017827	.0295

If we disregard all theoretical considerations and proceed by repeated trials to find what constants most nearly fit the experimental curves we get the values given in Table I. If we construct curves using these constants we get the results given in Table V.

In Fig. 3 and in Figs. 6–9 are shown curves expressing the observed values, the values calculated from constants observed by trial and the values calculated on a theoretical basis.

TABLE V.

OBSERVED VALUES COMPARED WITH VALUES CALCULATED FROM CONSTANTS OBTAINED BY TRIAL.

Per Cent. of Net Resistance in

Time in Hours	NaCl		98 NaCl + 2 CaCl₂		95.24 NaCl + 4.76 CaCl₂		85 NaCl + 15 CaCl₂		65 NaCl + 35 CaCl₂		38 NaCl + 62 CaCl₂		CaCl₂	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
1	40.21	41.62	103.1	103.6	108.2	106.3	124.5	115.3	136.1	123.7	148.1	127.2	154.0	152.9
2	22.82	20.74	103.8	105.6	112.1	110.3	126.1	124.1	141.9	136.2	149.0	141.7	152.1	148.9
3	14.54	13.65	105.8	106.3	112.1	112.6	128.5	128.8	143.2	141.9	149.0	147.9	143.2	136.5
4	11.28	11.24	106.1	106.3	113.9	113.8	130.2	130.9	143.9	143.8	149.0	149.7	128.8	123.8
5	10.30	10.43	106.1	105.8	113.1	114.1	130.2	131.3	143.7	143.3	149.0	148.8	117.2	112.2
6			104.9	105.0	113.1	113.9	126.5	130.6		141.5	136.1	146.5	101.8	101.8
10			102.1	100.5	107.9	110.2		123.3	129.5	129.6		132.6		
25			76.89	82.28	95.21	91.00	96.40	92.08	87.85	87.84	78.21	86.30	67.76	69.59
50			63.90	59.45	62.50	66.08	58.11	57.55	47.81	47.83	46.34	44.46	23.90	21.79
80			38.90	41.36	42.52	46.09	33.92	34.69	26.01	25.92	27.11	23.27	11.0	10.79
100			31.80	33.15	35.83	36.90	24.01	25.96	17.33	18.94	14.42	17.03	11.0	10.07

The measurements were made at 15° C. or corrected to this figure. Each experimental figure is the average obtained from 6 series of experiments.

In Table V. the calculated values necessarily show a better agreement with observed values than those in Table III., but even in the latter the difference between calculated and observed values is surprisingly small. The closeness of the agreement furnishes the most
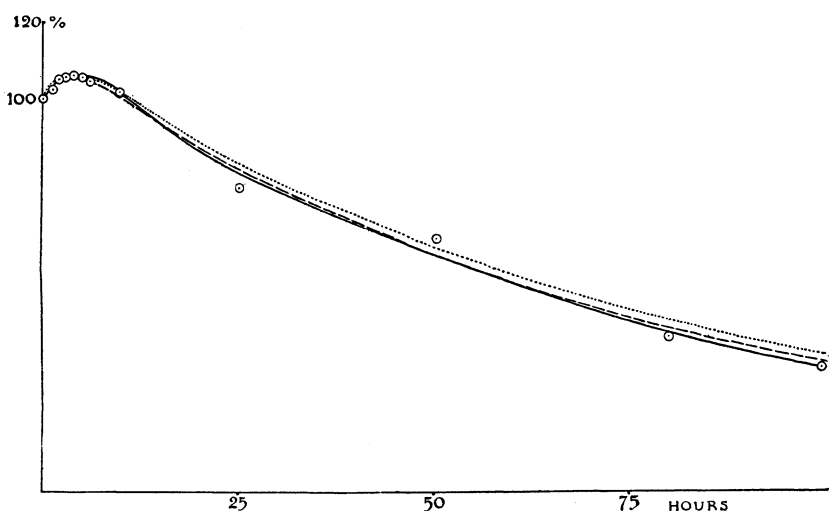
FIG. 6. Curve of electrical resistance of *Laminaria* in 98 NaCl + 2 CaCl₂ (—————), the trial curve (— — — — —) calculated from the velocity constants $K_1 = .000253$ and $K_2 = .00672$, and the theoretical curve (..........), calculated from the velocity constants .000241 and .00635.
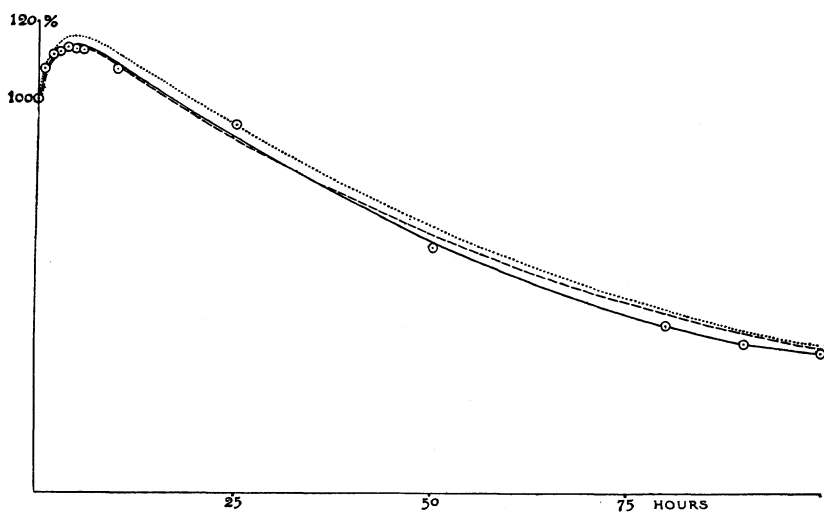

FIG. 7. Curve of electrical resistance of *Laminaria* in 95.24 NaCl + 4.76 CaCl₂ (—————), the trial curve (— — — — —) calculated from the velocity constants $K_1 = .000245$ and $K_2 = .00590$, and the theoretical curve (..........) calculated from the velocity constants $K_1 = .000240$ and $K_2 = .00566$.

striking proof of the essential soundness of the theoretical views on which the calculations are based.

It should be noted that while the values of $K_1 \div K_2$ for NaCl and for $CaCl_2$ fit the general scheme, the absolute values of K_1 and K_2 in these cases are much greater than we should expect. This shows that in pure $CaCl_2$ something accelerates both $A \rightarrow M$ and $M \rightarrow B$ to an equal degree (*i. e.*, multiplies both K_1 and K_2 by the
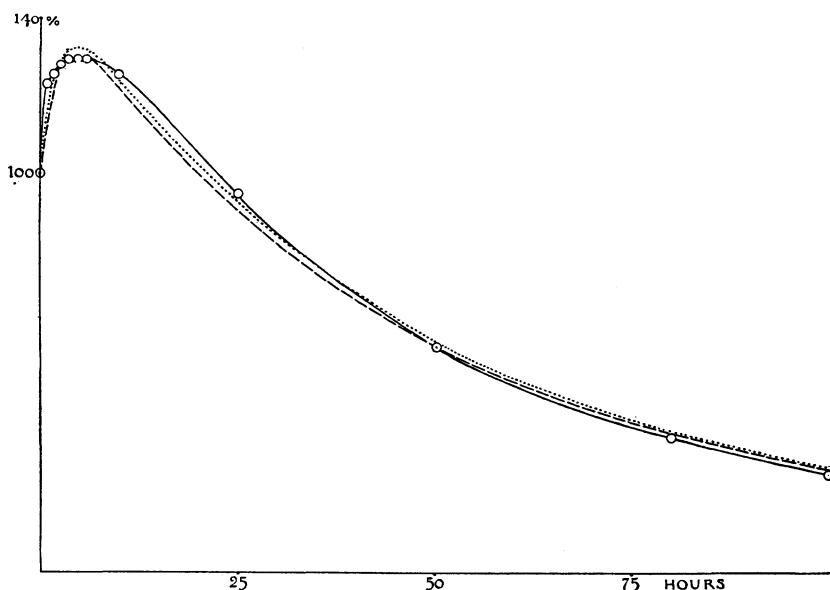

FIG. 8. Curve of electrical resistance of *Laminaria* in 85 NaCl + 15 $CaCl_2$ (————), the trial curve (— — — — —) calculated from the velocity constants $K_1 = .000364$ and $K_2 = .00730$, and the theoretical curve (..........) calculated from the velocity constants $K_1 = .000360$ and $K_2 = .00712$.

same factor); the same is true of NaCl, but here the acceleration is much greater than in $CaCl_2$. This acceleration might be due to a variety of causes and it seems unncessary to discuss it more fully at this time.

Figs. 10 and 11 show the curves of resistance in NaCl and in $CaCl_2$ and the curves calculated from constants obtained by trial.

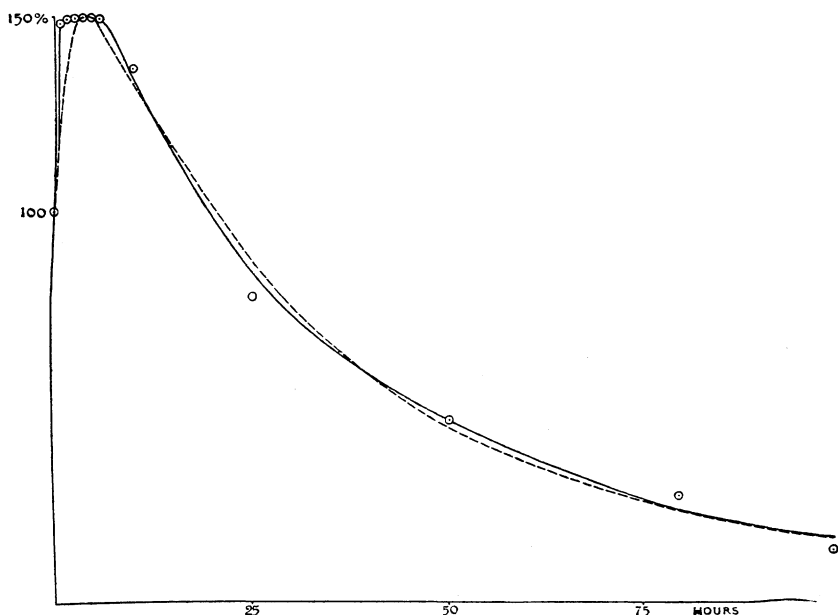Let us now consider briefly an alternative explanation of these results.

FIG. 9. Curve of electrical resistance of *Laminaria* in 38 NaCl + 62 CaCl$_2$ (————), and the trial curve (— — — — —) calculated from the velocity constants $K_1 = .000530$ and $K_2 = .0090$.
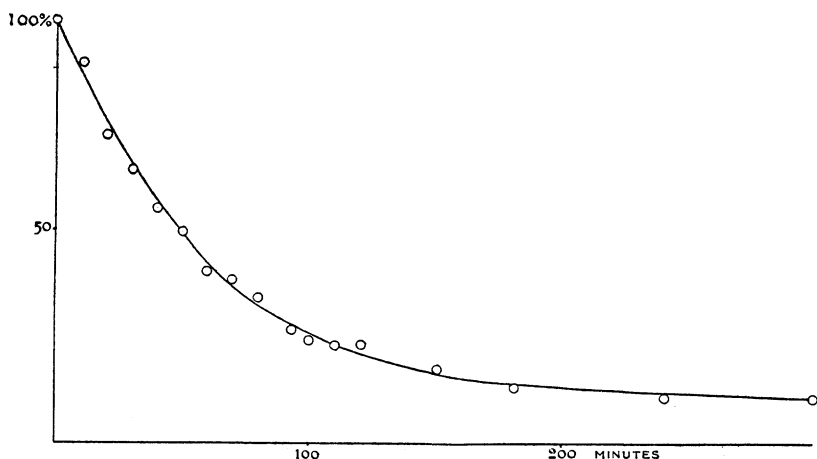

FIG. 10. Curve of electrical resistance of *Laminaria* in NaCl (........), and the trial curve (————) calculated from the velocity constants $K_1 = .018$ and $K_2 = .540$.

It might be assumed that no salt compound is formed but that K_1 is increased by $CaCl_2$ and that the effect is at a minimum at NaCl 95.24 + $CaCl_2$ 4.76. In this case K_1 would be very large in pure NaCl, very small in NaCl 95.24 + $CaCl_2$ 4.76 (or thereabouts) and would steadily increase as the proportion of Ca increased. If K_2 is increased by NaCl, with a minimum at NaCl 95.24 +
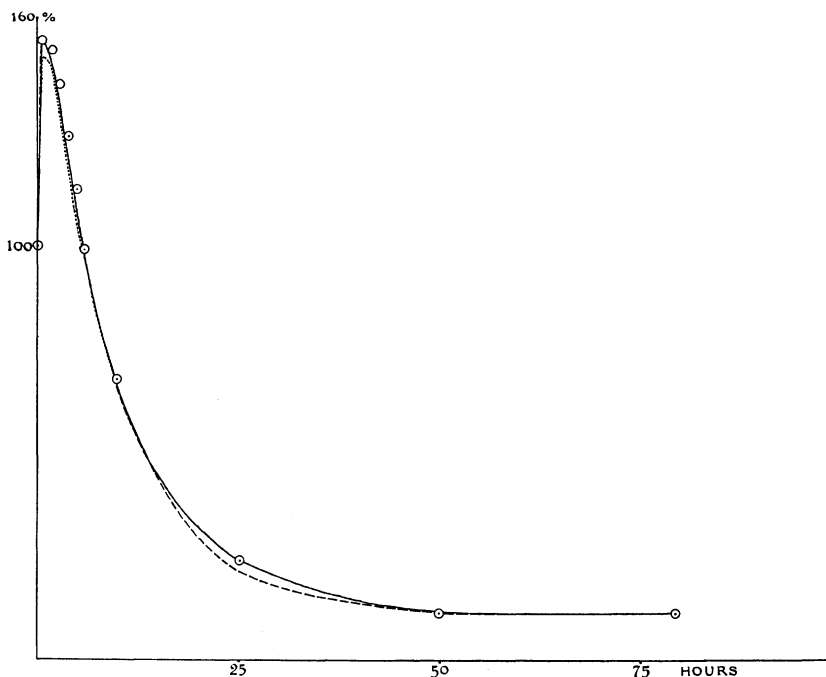

FIG. 11. Curve of electrical resistance of *Laminaria* in $CaCl_2$ (————), the trial curve (— — — — —) calculated from the velocity constants $K_1 = .0018$ and $K_2 = .0295$, and the theoretical curve (..........) calculated from the velocity constants $K_1 = .0017827$ and $K_2 = .0295$.

$CaCl_2$ 4.76, K_2 would be large in pure NaCl, would diminish as $CaCl_2$ was added until it reached a minimum at NaCl 95.24 + $CaCl_2$ 4.76 and would then rise steadily as more $CaCl_2$ was added.[14]

In this case it makes little difference whether the value of the

[14] It might also be assumed that both K_1 and K_2 are increased by $CaCl_2$ (or both by NaCl) with a minimum effect at NaCl 95.24 + $CaCl_2$ 4.76 But if only one salt controlled both K_1 and K_2 it would make little difference what the other salt was and this does not fit the facts.

constant is increased by the salt, the effect passing through a mini-
mum, or diminished by the salt, the effect passing through a
maximum.[15]

That catalyzers actually do act in this way is seen when acid
catalyzes lipase, the effect of the acid passing through a maximum.
It was found by Hoyer[16] that when oxalic or sulphuric acid is added
to lipase the action of the enzyme was accelerated in proportion to
the amount of acid but that above a certain concentration the effect
rapidly fell off.

Future investigations must decide which explanation fits the
facts in the majority of cases.

We have thus reached a quantitative explanation of the results
of our experiments. Our explanation indicates that the resistance
is proportional to the amount of M which is present in the proto-
plasm. It may therefore be of interest to enquire how the substance
M determines the resistance. If it is produced at the surface, as
we assume, it may form a layer in the surface which offers resist-
ance to the passage of ions. The amount of the resistance would
then depend on the thickness (and continuity) of the layer. This
may also apply to the internal surfaces, such as those of the nucleus,
plastids, vacuoles, and microsomes.

It is evident that whether or not any of these assumptions be
accepted certain facts seem to be established. It is clear that there
are two processes, one of which produces a rise, the other a fall
of resistance, and that their speed may be regulated by NaCl and
CaCl₂. It is extremely probable that these salts enter into chemical
combination with some constituent of the protoplasm and it is evi-
dent that the compound thus formed might regulate the speed of
one or both of these processes.[17]

[15] In all these explanations it is understood that NaCl and CaCl₂ act in a
saturated surface as explained above, so that only the relative proportions
are important.

[16] Hoyer, E., *Ber. deutsch. chem. Ges.*, 37(2) : 1436, 1904.

[17] It would seem that we can imitate completely the variations in re-
sistance which occur in living protoplasm only when we have a system in
which a reaction is going on which results in the production of a substance
which is comparable to that designated as M in the foregoing discussion.
Perhaps this explains why attempts to imitate these effects by experiments on
colloids have not been more successful.

It is clear that such consecutive reactions as are here discussed must be the rule in living matter and that further study of their dynamics should yield facts of importance.

SUMMARY.

I. Data regarding the electrical resistance of living tissues of *Laminaria* in NaCl .52 *M,* in CaCl$_2$ .278 *M,* and in mixtures of these are given.

II. In order to explain these data it is assumed:

1. That the resistance is determined by a substance M which is formed and broken down by the reaction

$$A \to M \to B.$$

2. That CaCl$_2$ accelerates the reaction $A \to M$.

3. That NaCl and CaCl$_2$ combine with a substance in the protoplasm, forming a compound which inhibits the reactions $A \to M$ and $M \to B$.

III. On the basis of these assumptions a theory is developed to explain:

1. Why both NaCl and CaCl$_2$ are toxic.

2. Why when mixed in the proper proportions their toxicity is greatly diminished (antagonistic action).

3. Why they have opposite effects on permeability.

4. Why the decrease of permeability produced by CaCl$_2$ must be followed by an increase when the exposure is sufficiently prolonged.

5. Why all toxicity disappears in sea water.

IV. The theory gives a quantitative explanation of the toxicity of all the mixtures and enables us *to predict the resistance (and permeability) in any mixture at any moment during exposure.*

V. The fact is emphasized that life processes must consist largely of consecutive reactions and that an analysis of the chemical dynamics of these reactions is indispensable for the solution of certain fundamental problems of biology.

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